

DO NATIVE ANTS PLAY A SIGNIFICANT ROLE IN THE REPRODUCTIVE SUCCESS OF THE RARE SAN FERNANDO VALLEY SPINEFLOWER, *CHORIZANTHE PARRYI* VAR. *FERNANDINA* (POLYGONACEAE)?

C. EUGENE JONES, YOUSSEF C. ATALLAH, FRANCES M. SHROPSHIRE, JIM LUTTRELL, SEAN E. WALKER, DARREN R. SANDQUIST, ROBERT L. ALLEN, JACK H. BURK, AND LEO C. SONG, JR.

Department of Biological Science, California State University, Fullerton, CA 92831  
cejones@fullerton.edu

ABSTRACT

Previous field studies of the reproductive biology of the San Fernando Valley spineflower, *Chorizanthe parryi* var. *fernandina* (S. Watson) Jeps. suggested that pollination by ants might be an important feature of this endangered polygonous taxon. This conclusion was based on observations that native ants were abundant floral visitors and constant to this species. We conducted the current study to explore more closely the possibility that native ants were facilitating pollination and resulting in viable seed set. Based on our data, ants can indeed be effective pollinators of spineflower. Fruit set was 57% higher in flowers exposed to ant visitation, compared to 27% in control flowers where ants were excluded. Further, a 25.7% germination rate was observed for achenes produced in the absence of ants, in contrast to a 61% rate in those produced in the presence of ants. We suggest that ant pollination may be more prevalent in drier climates, ant production of inhibitory substances may not be a severe limitation to their function as pollinators, invasive Argentine ants may pose a threat to plants pollinated by ants, and self-pollination may not be a negative attribute for ant pollinated plants.

Key Words: Allee effect, ant pollination, bet-hedging, *Chorizanthe parryi* var. *fernandina*, mixed mating strategies, Polygonaceae, San Fernando Valley spineflower, selfing.

Our recent studies have investigated a variety of factors related to the reproductive success of *Chorizanthe parryi* S. Watson var. *fernandina* (S. Watson) Jeps., the San Fernando Valley spineflower (SFVS), an endangered California species formerly thought to be extinct (Jones et al. 2009, C. E. Jones unpublished report<sup>1</sup>). These studies have demonstrated that the SFVS can self-pollinate and possesses a general mixed mating strategy. Abundant fruit is set and it is visited by a variety of potential pollinators, including native ants. Indeed, ants are among the most frequent visitors to the flowers of the SFVS (Jones et al. 2009). This finding prompted the current investigation into whether or not ants facilitate successful pollination and fruit set for this species.

Ants are rarely considered to be effective pollinators (Hölldobler and Wilson 1990; Peakall et al. 1991) due to their small size, which can allow them to maneuver in and out of flowers without contacting anthers or stigma (Faegri and van der Pijl 1979; Inouye 1980), their smooth bodies, which are not well suited to pollen transport (Schubart and Anderson 1978; Puterbaugh 1998), and chemical secretions from the metapleural gland that reduce pollen viability, germination, and pollen tube growth (Beattie et al. 1984; Gómez and Zamora 1992). These chemical secretions

contain myrmicacin (3-hydroxydecanoic acid, a broad-spectrum antibiotic), which has been shown to disrupt the flow of components to cell wall formation, the function of Golgi vesicles, and mitosis (Iwanami and Iwaware 1978; Nakamura et al. 1982), thereby affecting pollen germination and pollen tube growth. In addition, ants groom themselves frequently, decreasing the likelihood of transferring pollen from one plant to another (Beattie et al. 1985).

Rico-Gray and Oliveira (2007) list sixteen plant species in which ant pollination has been demonstrated including two well-documented cases of ant pollination by Peakall et al. (1991) and Puterbaugh (1998). Hickman (1974) previously noted ten adaptive characteristics commonly shared by such ant-pollinated plants: 1) plants are found in hot and dry climates where ant activity is high; 2) nectaries are accessible to short-tongued insects; 3) plants are short or prostrate; 4) there are dense populations of plants with interdigitating branches; 5) few blooms occur at once per plant; 6) the flowers on erect plants are sessile or are found on the surfaces of low-growing matted plants; 7) pollen volume per flower is small; 8) few seeds are produced per fruit; 9) flowers are small with minimal visual attraction; and 10) small amounts of nectar are produced.

Hickman (1974) developed this list during his studies on *Polygonum cascadenense* W. H. Baker (Polygonaceae), an ant-pollinated annual found in the hot dry climate of the Western Cascades of

<sup>1</sup>Unpublished reports by CEJ are available upon request from the senior author.

Oregon. In populations where the ants were abundant, he showed that plants had 85–100% seed set, whereas greenhouse plants, exposed to flying pollinators only, exhibited 0–7% seed set. Hagerup (1932), Faegri and van der Pijl (1979), and Rico-Gray (1989) have also noted that ant pollination is more likely to occur in dry and hot climates, where flying pollinators often are not abundant. Small flowers growing near to the ground with minimal visual attractants may also be associated with ant pollination (Faegri and van der Pijl 1979; Gómez et al. 1990a, b; Garcia et al. 1995; Proctor et al. 1996).

Also, among the described examples of an ant-pollinated species is *Ptilotrichum spinosum* (L.) Boiss. (Brassicaceae), a low-growing, woody plant found in the Sierra Nevada of southern Spain (Gómez et al. 1990a, b). This species bears small hermaphroditic flowers that are frequently visited by ants and exclusion experiments were employed to study the possible effects of such visits.

During our previous investigations, we noted that the SFVS appears to share many of the characteristics observed in these published studies, with the exception of number 5 (few blooms occur at once per plant) on Hickman's (1974) list. Furthermore, the diameter of the SFVS floral tube is only slightly larger than the head widths of the native ants frequently found visiting the plant (C. E. Jones unpublished report), a characteristic noted as important by L. LaPierre (unpublished report). Additionally, these native ants were observed moving in and out of the flowers and did contact both the anthers and stigma in the process (C. E. Jones unpublished report), supporting the suggestion that ants may indeed be significant pollination vectors of the SFVS.

Based upon the high visitation rates documented by Jones et al. (2009, C. E. Jones unpublished report) and Wyatt and Stoneburner (1981), we predict that a significantly higher fruit-set will occur in the plants exposed to ant vectors compared to plants where all vectors are excluded. A previous study showed that a fruit set of about 25% occurs even when all pollination vectors are excluded (Jones et al. 2009, C. E. Jones unpublished report). A significantly higher fruit-set in the ant vector exposed group than that found in the absence of all pollinators would strongly suggest that ant species, specifically *Dorymyrmex insanus*, play a significant role in the pollination biology of the SFVS.

Since at least three different species of native ants were very common visitors to the flowers of the SFVS at the Ahmanson and Newhall Ranch sites (Jones et al. 2009), we decided that follow-up studies were warranted to address the following questions: 1) Is the SFVS adapted for ant pollination? 2) Do ants serve as effective pollinators in the absence of other vectors? 3) Is any seed produced by ant pollination viable? 4) Can this

species self without a vector? 5) What are the possible evolutionary implications of ant pollination for the SFVS?

## MATERIALS AND METHODS

### Plant Species

Formerly distributed in Southern California from Lake Elizabeth in Los Angeles Co. to near Del Mar in San Diego Co. (Munz and Keck 1959; C. E. Jones unpublished report; Glenn Lukos Associates, Inc. unpublished report), *Chorizanthe parryi* var. *fernandina*, the San Fernando Valley spineflower (SFVS) is an herbaceous annual found within coastal sage communities at elevations below 350 m (Munz and Keck 1959; C. E. Jones unpublished report). After being considered extinct for a time (Hickman 1993), the SFVS has been found in two locations (the Ahmanson and Newhall Ranches – sites of our previous studies), where it occurs primarily in dry, sandy places within coastal sage in dense patches of several hundred plants (CNDDDB 2001). It is currently designated as a List 1B.1 plant (Rare, Threatened, or Endangered in California or Elsewhere; seriously endangered in California) by the California Native Plant Society and is State-listed Endangered (CNPS 2001) and a Federal candidate for similar listing (CNPS 2005).

Stems of the SFVS mostly spread horizontally from the base to form a low, flat-topped, grayish plant 0.2–0.8 (1) dm high and 0.5–4 (6) dm across (Jepson 1925; Reveal 1989). The predominantly sessile, single-flowered involucre are more or less openly distributed in small clusters (Munz and Keck 1959) at branchlet ends (Jepson 1925) and are urn-shaped, bearing six bracts and three awns (Reveal 1989; see Fig. 1). These involucre awns are straight rather than hooked in the SFVS, a trait that distinguishes *C. parryi* var. *fernandina* from the more widely distributed *C. parryi* var. *parryi* (Reveal 1989).

The sessile flowers are 2.5–3 mm long with a greenish-white tube and six white, sparsely hairy lobes, occurring in two series of three (Reveal 1989; Hickman 1993). Nectar is present around the base of the ovary and between the filaments. The flowers are protandrous (Taylor-Taft 2003) and are produced in late spring, April–June (Munz and Keck 1959). Voucher SFVS specimens were deposited in the Fay A. MacFadden Herbarium (MACF) at California State University, Fullerton, CA.

### Ant Species

The small, medium brown pyramid ant, *Dorymyrmex insanus* (Hymenoptera: Formicidae; Dolichoderinae) was selected as the model ant for our investigations since it was the dominant ant



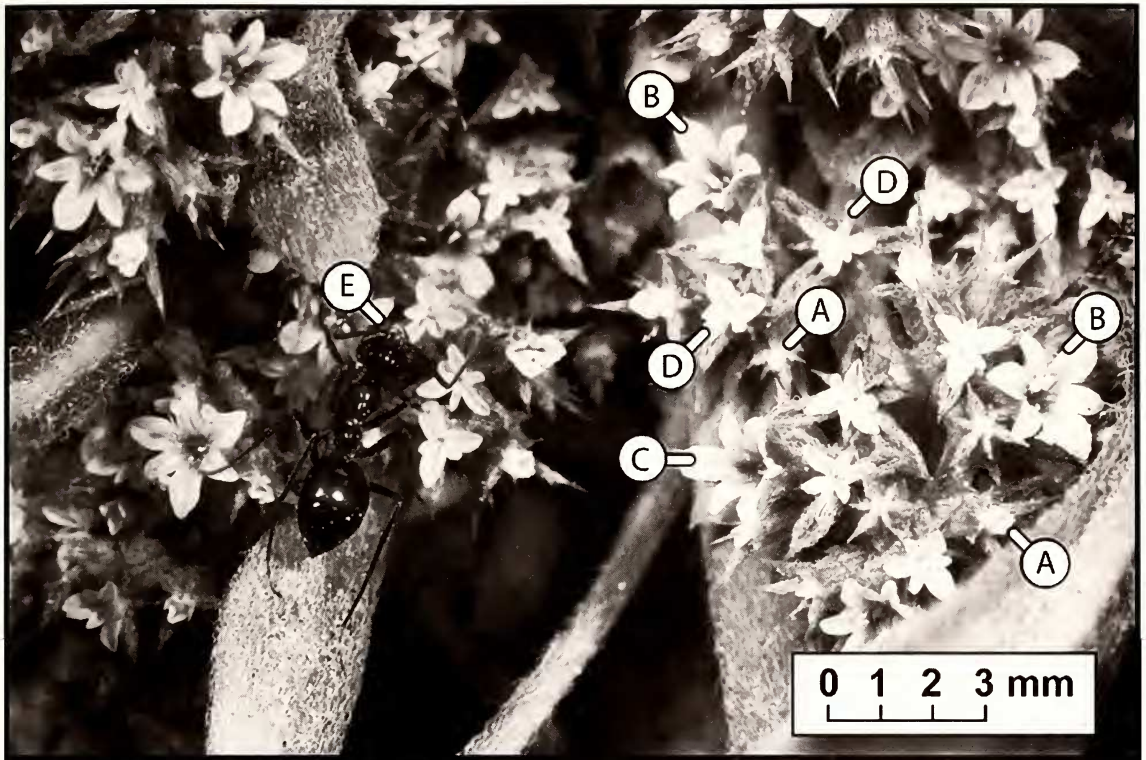


FIG. 1. Photo of *Chorizanthe parryi* ssp. *fernandina* with pollinator. (A) Unopened flower bud. (B) Open flower with dehiscent anthers – flowers protandrous. (C) Open flower with receptive stigma. (D) Post-pollinated flower – perianth retained. (E) Pollen on head of Pyramid Ant, *Dorymyrmex insanus*. Photo by Robert L. Allen.

visitor to the SFVS flowers at the Ahmanson Ranch (C. E. Jones unpublished report) and, on our preliminary visits to the Newhall Ranch, colonies of this ant were found near, although not visiting, the populations of the SFVS that we subsequently investigated there (C. E. Jones unpublished report).

Snelling (1995) describes the *Dorymyrmex insanus* worker neotype measurements as: head length 0.90 mm, head width 0.79 mm, scape length 0.87 mm, eye length 0.26 mm, and total length 3.1 mm. Most importantly, Snelling (1995) further notes that the entire head (except the clypeus, frontal area and gena), mesosoma and gastral terga are pubescent, which would provide a surface where pollen grains could adhere. These ants are predaceous, but are also attracted to sugary substances like honeydew and, presumably, nectar (Wheeler and Wheeler 1973).

#### Experimental Design

This controlled study was carried out between May and July of 2004 and between May and July of 2005 and consisted of two enclosures each composed of two sections. The top portion, measuring 91.44 cm × 30.48 cm × 10.16 cm, consisted of four pine boards and a 1.59 mm

hardware mesh top allowing the entry of light. The bottom of each enclosure consisted of four pine boards and a plywood base of equal size to the top and contained approximately 4 in. of Sta-Green Premium Container Mix with Fertilizer (United Industries, Atlanta, GA) with more or less 7 mm of commercially available sterile sand spread evenly over the entire surface area. As an added precaution, these screened enclosures were chemically treated using “Cooks Ant Barrier”, a commercially available insect repellent. The sides of the enclosures were initially sponged with the solution until the wood was heavily saturated and this treatment was repeated at regular intervals (Luttrell 2006).

SFVS achenes were extracted from plants collected from the Ahmanson Ranch site 3 (34°25.12'N, 118°35.14'W) during the first week of April 2004 and held in refrigerated storage until removal from the inflorescences. The inflorescences were first wetted with de-ionized water and placed in a strainer, where rotational hand pressure was applied to the wet clumps and the loosened seeds settled into a bowl of de-ionized water. The collected achenes were then rinsed five times again with de-ionized water in order to leach out any possible chemical germination inhibitors (Luttrell 2006).

The extracted achenes were divided into two equal groups and each group (approximately 100 fruits per group) was evenly planted in a black plastic tray (43.5 cm square  $\times$  60 mm deep) filled with same premium grade potting soil as before and top layered with the same commercially produced sterile sand. Planting was completed during the last week of April 2004 and 2005. Subsequent germination took place in a controlled environment (see details below) and began to occur after 9 d and was considered complete after 14 d. In 2004, seedlings were allowed to grow and resulted in 32 seedlings surviving in one flat and 27 in the second flat. In 2005, seedlings were allowed to grow until overcrowding prompted the removal of all but 40 plants per container.

The SFVS plants were next placed in the two separate screened enclosures, 32 plants in Enclosure 1, and 27 in Enclosure 2. Finally, the plants, trays and enclosures were placed in an indoor controlled setting equipped with timed fluorescent lighting (grow lamps, T-12, 40 watt, Sylvania fluorescent tubes, approximately 61 cm long).

#### Pollination and Fruit Production Observations

For each trial, approximately 500 sterile female *Dorymyrmex insanus* worker ants were collected from the Ahmanson Ranch site 3 (34°25.12'N, 118°35.14'W) at the end of the second week of May 2004 (first trial) or the second week of May 2005 (second trial) and were introduced into Enclosure 1 – Experimental, whereas no vectors were introduced into Enclosure 2 – Control. The screening and chemical treatment, combined with the indoor controlled setting, ensured that no outside pollination vectors would be capable of entry. This extra precaution was taken to rule out the possibility that some smaller flower-visiting potential pollinators might be able to gain access to the flowers in Enclosure 2. Additionally, the chemical treatment prevented the escape of the introduced potential ant pollination vector, *Dorymyrmex insanus*.

In each trial, the number of flowers on each plant and subsequent seed set per flower were counted in both enclosures. After all plants had died in each of the two enclosures, each individual plant was harvested and seeds from these plants were removed and counted. To assess seed viability, three hundred achenes from Enclosure 1 – Experimental (with ants) and three hundred from Enclosure 2 – Control (with no potential vectors) were each divided into replicates of 15 achenes each and placed on moistened 38 lb., 8.9 cm circles of regular seed germination paper (Anchor Paper Company, St. Paul, MN) in 100  $\times$  15 mm Fisherbrand disposable sterile Petri dishes (Fisher Scientific, Los Angeles Office, Tustin, CA). A total of 40 Petri dishes (replicates) were utilized, with 15 achenes from each enclosure.

Each Petri dish was watered with 5 ml of deionized water and placed in an individual Ziploc® one quart storage bag (S. C. Johnson & Sons, Inc., Racine, WI) and randomly placed in one of four Percival Model E-30B growth chambers (Percival Scientific, Inc., Perry, IA). Each growth chamber was then programmed for 11 hr of daylight with 15C daytime temperature and 10C nighttime temperature. Germination was monitored and recorded for each Petri dish replicate daily from 12 December 2005 through 6 January 2006.

#### Statistical Analysis

Data on post-experimental seed set were analyzed using a paired-t test (Excel). Data from all 40 replicates from the seed viability experiment were pooled into those produced with ants (20 replicates) versus those produced without ants (20 replicates) and then were compared using a chi-square goodness of fit test.

### RESULTS

#### Pollination and Fruit Production

We found a significant difference between fruit set in control and pollinator enclosure treatments in 2004 ( $P < 0.001$ ,  $t = 20.387$ ,  $df = 38$ ) and 2005 ( $P < 0.001$ ,  $t = 24.612$ ,  $df = 38$ , Table 1). Approximately 22% of the SFVS flowers within the control group, which lacked ants, set fruit without a vector as compared to 78% fruit-set in the Experimental flowers exposed to the ant species *Dorymyrmex insanus*. Fruit set in the presence of the ant *Dorymyrmex insanus* is significantly higher by approximately 56% in the 2004 trial, and is also significantly higher by about 57% in the 2005 trial (Table 1). These differences in seed set occurred despite the fact that the number of flowers produced per plant was not significantly different for the 2004 trial ( $P < 0.05$ ,  $t = 0.82$ ,  $df = 1$ ) or for the 2005 trial ( $P < 0.05$ ,  $t = 0.28$ ,  $df = 1$ ), Table 1).

We found significant differences in germination under controlled growth chamber conditions of achenes harvested from each enclosure experiment (STATS). Of the 300 achenes produced in control Enclosure 2 (i.e., in the absence of any vector, 77 seeds germinated (25.7% germination). In contrast, of the 300 achenes produced in Experimental Enclosure 1 (i.e., in the presence of ants), 183 germinated (61% germination). Achenes produced without a vector were less than half as likely to germinate than those produced in association with ant vectors ( $\chi^2 = 43.22$ ,  $P < 0.01$ ,  $df = 1$ ).

### DISCUSSION

In terms of pollination interactions, of the ten characteristics noted by Hickman (1974) in his



TABLE 1. COMPARISON OF NUMBER OF FLOWERS PRODUCED PER PLANT AND FRUIT SET PER PLANT FOR THE TWO ENCLOSURES FOR THE 2004 AND 2005 TRIALS. Difference in percent fruit set is significant at  $P < 0.001$  for both 2004 and 2005 trials ( $t = 20.387$ ,  $df = 38$ ) for the 2004 trial and ( $t = 24.612$ ,  $df = 38$ ) for the 2005 trial. Note: difference in average number of flowers per plant is not significant for either trial year, ( $P < 0.05$ ,  $t = 0.82$ ,  $df = 1$  for 2004 and  $P < 0.05$ ,  $t = 0.28$ ,  $df = 1$  for 2005).

| Character   | Total produced on all plants | Range per plant (in %) | Average per plant (in %) | Standard deviation per plant |
|---|------------------------------|------------------------|--------------------------|------------------------------|
| Enclosure 1 with ants present. (n = 32 for 2004 trial vs. n = 40 for 2005 trial). |                              |                        |                          |                              |
| Number of flowers produced – (2004)   | 2977                         | 49–132                 | 109.1                    | 23.6                         |
| Number of fruit produced – (2004)   | 1922                         | 51–97                  | 78.3                     | 15.2                         |
| Number of flowers produced – (2005)   | 4422                         | 51–144                 | 110.6                    | 22.5                         |
| Number of fruit produced – (2005)   | 3728                         | 55–100                 | 84.3                     | 13.8                         |
| Enclosure 2 without ants present. (n = 27 for 2004 trial vs. 40 for 2005 trial).  |                              |                        |                          |                              |
| Number of flowers produced – (2004)   | 2480                         | 42–138                 | 103.9                    | 24.7                         |
| Number of fruit produced – (2004)   | 723                          | 14.5–33.8              | 22.1                     | 6.3                          |
| Number of flowers produced – (2005)   | 4481                         | 44–142                 | 112.0                    | 22.3                         |
| Number of fruit produced – (2005)   | 1196                         | 16.8–38.5              | 26.7                     | 5.2                          |

description of what has been termed the “ant pollination syndrome” (Hickman 1974; Faegri and van der Pijl 1979), all except number 5 (few co-occurring blooms per plant) were fulfilled in the SFVS. What we found in the SFVS may significantly increase the floral display, which would seem to be adaptive for attracting a diverse group of flying insects that we found (Jones et al. 2009). Post-pollination retention of the perianth (see Fig. 1) also serves to increase the floral display (Jones and Cruzan 1999).

In other studies, such as those by Garcia et al. (1995) on *Borderea pyrenaica* Miégev. (Dioscoreaceae) and Mayer and Gottsberger (2002) on *Arenaria serpyllifolia* L. (Caryophyllaceae), there was substantial or complete congruence with all ten tenets postulated by Hickman (1974) as characteristic of myrmecophilous species.

Data from our previous observational surveys clearly support the hypothesis that ants play a significant role in the pollination biology of this taxon (Jones et al. 2009, C. E. Jones unpublished report). In terms of the entire flowering season, ants accounted for 51% of visitors and 37% of visits in the Ahmanson Ranch study (C. E. Jones unpublished report) and 21% of the visitors and 34% of the visits in the Newhall Ranch study (C. E. Jones unpublished report). Ants were especially important during early and late portions of the season at the Ahmanson Ranch (C. E. Jones unpublished report), providing 84% of the visitors and 54% of visits during the former period and 77% of visitors and 71% of visits during the latter. More visits were made by the ant species *Dorymyrmex insanus* (3711 of 9830 or 38%) than by any other ant taxon. Seasonal mean number of SFVS flowers visited per *Dorymyrmex* individual per observation period was 3.1 in that study. A second ant species, *Solenopsis xyloni*, was also a prominent visitor albeit in much smaller numbers (257 visits) (C. E. Jones

unpublished report). Yet a third species of ant (*Forelius mccooki*) was an important visitor at the Newhall Ranch (C. E. Jones unpublished report).

Photographic evidence (see Fig. 1 – note pollen being carried by the individual of *D. insanus*) and SFVS pollen removed from collected ant specimens support visual observations that pollen is indeed being carried by ant visitors (C. E. Jones unpublished report).

Although we examined a relatively small sample of ants captured on SFVS flowers at the Ahmanson Ranch, those that were examined had pollen loads that were 98% specific to the flowers of the SFVS, indicating that individual ants were purposefully visiting these plants for nectar and in the process, picking up pollen and very likely facilitating the successful reproduction of the SFVS (C. E. Jones unpublished report). Jones and colleagues (C. E. Jones unpublished report) found that the ant species *Forelius mccooki* visited SFVS flowers at the Newhall Ranch (C. E. Jones unpublished report) and showed that 13 of 17 individuals collected on SFVS flowers carried one or more pollen grains of the SFVS. Further, of the 13 that carried pollen, nine bore only SFVS pollen and were, therefore, deemed 100% constant to the SFVS. The remaining four specimens carried mixed loads but all included some SFVS pollen (C. E. Jones unpublished report).

We demonstrated that individuals of the ant species *Dorymyrmex insanus* were effective pollen transporters and facilitated fruit-set in over 50% more flowers than in the case where all pollinators were excluded. Furthermore, ants move extensively among flowers on any given plant and, in doing so, likely promote geitonogamy. Therefore, it is highly likely that the ants promoted an increase in overall selfing, as well. However, ants also move between flowers on different plants when those plants are in close proximity to one another. In doing so, ants

facilitate xenogamy. Therefore, ants very likely do facilitate some level of out-crossing, though that level is likely to be much less than a bee pollination vector like the honey bee. The percentage of viable fruit, as judged by germination of the SFVS achenes produced by ant visitation, was 61% - over double the germination rate found for achenes produced in flowers by selfing without a vector.

The potential capacity to set seed in the absence of any pollination vector further increases the likelihood of successful SFVS reproduction. A fruit-set of approximately 33% is normally expected for out-crossing plant species (Sutherland 1986). Values above that number are suggestive of a plant that is at least a facultative selfer. Given a fruit set close to 60% in the SFVS and given the small size of the flowers, the SFVS would seem to be a facultative selfer. However, it is unlikely that it would be an obligatory selfer since the flowers are protandrous.

The occurrence of selfing without a vector within a single flower might not be possible unless the pollen remains viable until the stigma is receptive and, in addition, the anthers are positioned close enough to the stigmatic surface to facilitate pollen transfer. Our data would indicate that at least some portion of the pollen does remain viable long enough to result in selfing without a vector. Further, the anthers are positioned close enough to the stigmatic surface to result in transfer of pollen without the necessity of a vector. Given the manner in which ants and other small insects move among the SFVS flowers, vector-assisted selfing should be considered likely.

Our data indicate that 27% of the seed set occurred within plants in which all potential pollinators were prohibited from visiting the flowers. The significantly lower number of fruits produced by the selfing treatment versus in the enclosure with ants indicates that the SFVS is not a productive selfer without a vector. However, 27% seed set is probably sufficient to ensure reproductive success of the SFVS in unfavorable years. Furthermore, in a germination test carried out on a single sub-sample of the seeds produced by selfing without a vector, approximately one third (47 of 150 or 31%) did germinate. Our data seem to indicate that achenes produced by selfing without a vector have a lower viability, as judged by germination rates, than achenes produced with the aid of ants. Unresolved, then, is the question of the viability and/or fertility of adult plants produced from such selfing. Our results support the postulate by Stebbins (1957) that geographically restricted plants are likely to be self-compatible.

Self-pollination or autogamy appears to be quite common in ant-pollinated species (examples include Wyatt and Stoneburner 1981; Gomez et

al. 1990a; Peakall and Beattie 1991; Gomez and Zamora 1992, 1999; Gomez et al. 1996; Bosch et al. 1997; Gomez 2000, 2002; Buide and Guitián 2002; and Kawakita and Kato 2002). Why is selfing so common in these taxa? In part, this may be attributed to the relatively short distances that crawling ants travel between flowers and to the fact that the frequency distributions of these visits are strongly leptokurtic (Wyatt and Stoneburner 1981).

As was pointed out by Gomez (2002) in his study of selfing in *Euphrasia willkommii* Freyn (Scrophulariaceae), an endemic alpine species of the Spanish Sierra Nevada, selfing may represent an "ecological mechanism to ensure successful reproduction in a harsh environment where pollinator availability is low." Affre et al. (1995) also suggested that a scarcity of pollinators, as well as fragmentation and isolation of populations, could increase the frequency of self-pollination in a Mediterranean endemic *Cyclamen*. We would only add that in unpredictable environments, such as Mediterranean ecosystems, where annual rainfall seems to be a major limiting factor, differences in annual survivorship can result in dramatic fluctuations in plant and pollinator densities. Small populations resulting from such unpredictable conditions are more likely to experience Allee effects due to pollen limitations caused by reduced mate availability (Groom 1998; Moeller 2004). This leads to uncertain reproductive success if the plant requires a pollination vector and, therefore, has important consequences regarding the population dynamics of the species (Clauss and Venable 2000).

In the SFVS, significant annual variation in population numbers has been recorded and reflects variation in seasonal rainfall (Dudek, Dudek and Associates, Inc., and Sapphos Environmental, Inc. unpublished reports). This variation in plant densities also seems to be associated with substantial variation in pollinator availability both in terms of species composition and total numbers (Jones et al. 2009, C. E. Jones unpublished report). Therefore, non-facilitated autogamy would appear to be functioning as a bet-hedging pollination strategy in arid regions, similar to the variation in seed germination strategies found in desert annuals (Clauss and Venable 2000). Such a strategy in the annual SFVS assures some successful fruit/seed set in the face of potentially reduced numbers of both plants and pollinators (reproductive assurance as discussed by Jarne and Charlesworth 1993). The preservation of genotypes that are well adapted to survival and reproduction under drought conditions may be another advantage of selfing in the SFVS (Jarne and Charlesworth 1993).

In this regard, it is interesting to note in Peakall and Beattie's (1991) study of ant-facilitated



selfing in the orchid *Microtis parviflora* R. Br. that, although their electrophoretic analysis indicated that the populations were highly inbred, some outcrossing was occurring. They indicated that the ant foraging witnessed on this species would have yielded a mixed mating system similar to those reported for a variety of other insect pollinators (e.g., Vogler and Stephenson 2001). Thus, in the case of a facultative selfer like the SFVS, ants appear to provide a reliable pollination vector that ensures successful reproduction via both selfing (fitness in times of stress) and outcrossing (production of genetic variation for possible adaptation to future environmental fluctuations).

Our data indicate that during harsh, dry, growing seasons, the SFVS may survive by producing a significant number of progeny via auto-fertility or by utilizing various native ant species as major pollination vectors. A decrease in the number of floral visitors or the production of a significant number of progeny via selfing with or without a vector would have important genetic implications in terms of inter-population gene flow (Ellstrand and Elam 1993; Jarne and Charlesworth 1993). A detailed analysis of the population genetics of this species throughout its extant range would help determine its genetic status and to establish management strategies to maintain or enhance population genetic diversity.

Although ants may not visit as many flowers per foraging bout as other pollinators, they are present in greater abundance and are clearly superior in facilitating pollination. In addition, ants tend to be consistently present throughout the flowering season in contrast to other visitors that often display more limited availability, frequently appearing only during the peak of the flowering season. Certainly this is the case in the SFVS (Jones et al. 2009). Further, ant-pollinated systems are low-energy systems allowing for a reduced energetic commitment (e.g., in the production of very small quantities of nectar per flower as was documented in the SFVS – Jones et al. 2009) on the part of the plant species (Hickman 1974). Such energetic savings could be extremely important to the survival of plants living in unpredictable environments and may aid in ensuring that the plant will have sufficient resources for at least some reproduction even in very dry years (Heinrich and Raven 1972; Svensson 1985).

A potential problem of concern for ant pollinated species like the SFVS is the invasive alien Argentine ant. Argentine ants (*Linepithema humile*) are considered to be among the top 100 worst invasive alien species globally (Lowe et al. 2000). A major practical concern related to ant-pollinated systems is the question of how interactions between invasive Argentine ants and native ants will affect the reproductive

biology of these plants (Lack 2003). Argentine ants have been shown to significantly reduce the foraging success of native ant species by being more efficient at exploiting food sources and thereby displacing native ant species from areas where they successfully invade (Human and Gordon 1996, 1997; Suarez et al. 1998). Although, Suarez et al. (1998) found that Argentine ants normally penetrated only approximately 100 m into Mediterranean type ecosystems in San Diego Co., CA, it is unclear whether the process of invasion is not yet complete or whether they were not penetrating into these habitats because they lacked water. Holway (2005) found that Argentine ants were able to move into coastal sage scrub habitats in southwestern San Diego Co., CA by using riparian corridors; however, their numbers decreased with increasing distance from anthropomorphic influences as they moved into drier scrub habitats.

Although Argentine ants apparently have not yet successfully invaded the drier areas in the Mediterranean ecosystems found in southern California, they have invaded and colonized other dry habitats in Hawaii (Cole et al. 1992; Reimer 1993), the fynbos in South Africa (Giliomee 1986; Lack 2007), and the matorral in Chile (Fuentes 1991), so they may very well eventually invade the similar dry habitats in Southern California. No data are currently available regarding whether or not Argentine ants could serve as an effective replacement for native pollination vectors for species like the SFVS. They may be less effective than the native ants because of their much smaller size, which might result in their not contacting both anthers (for pollen pickup) and/or stigmas (for pollen deposition) in the SFVS. Clearly, the issue of potential Argentine ant impacts on the reproductive biology of plants like the SFVS, which seems to depend on ants for a significant part of their reproductive effort, requires further investigation.

We show that native ants are effective pollinators of SFVS, transporting pollen between flowers and leading to significant increases in seed set and seed viability, compared to when ants are excluded from flowers. Taken together, our results provide evidence that although the SFVS can set fruit without a vector, fruit set in the presence of the ant *Dorymyrmex insanus* is significantly higher. Results from the current investigation contribute significant information to the SFVS database and should prove useful to conservation biologists charged with protecting this rare taxon.

Based on our study and a review of the literature, we stress the importance of investigating the reproductive biology of other plants found in dry, harsh, variable environments and predict that many more species will be found to receive a significant portion of the pollen

deposited on their stigmatic surfaces via ant vectors. Thus, a better understanding of the role of ants in the successful reproduction of native plants of dry environments is crucial to our comprehension of how these communities function and the ecological tradeoffs made under the variable and unpredictable environmental conditions that prevail in such habitats.

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